

DETECTION OF *MYCOBACTERIUM BOVIS* IN CAPTIVE SLOTH BEARS (*MELURSUS URSINUS*) BY POLYMERASE CHAIN REACTION

**M. VEERASELVAM¹, R. SRIDHAR², T. M. A. SENTHILKUMAR³, M. G. JAYATHANGARAJ⁴ &
P. PERUMAL⁵**

^{1,2,3,4}Department of Wildlife Science, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University,
Chennai, Tamilnadu, India

⁵National Research Centre on Mithun (ICAR), Jharnapani, Nagaland, India

ABSTRACT

Diagnosis of Mycobacterial infection is paramount important from the public health perspective since treatment and control measures are very significant, particularly in captive animals. In this diagnostic study of *Mycobacterium bovis* infection in sloth bears (*Melursus ursinus*), polymerase chain reaction (PCR) had been used with the primer sequence of *pncA*-8 (5'-GGTTGGGTGGCCGCCGGTCAG-3') and *pncA*-11 (5'-GCTTTGCGGCGAGCGCTCCA-3') that were specific for *Mycobacterium bovis* *pncA* gene. Forty two fresh faecal samples were collected randomly from the apparently healthy sloth bears maintained in captive conditions. The DNA extraction procedure was done as per the manufacturer's protocol and further subjected to amplification. The amplification profile includes respectively: initial heating of the samples for 5 minutes at 94°C, annealing at 55°C for 1 minute, primer extension at 72°C for 1 minute and final elongation step for 10 minutes at 72°C. Out of 57 samples, 5 samples were yielded on expected amplified PCR product size of 744 bp when electrophoresed in 1.5% agarose gel. A positive control of *Mycobacterium bovis* DNA procured from Tuberculosis Research Centre and a negative control from a healthy bovine sample were used. These results demonstrated that PCR test will increase the effectiveness of laboratory diagnosis to detect and identifying the *Mycobacterium bovis* in captive wild animals.

KEYWORDS: Sloth Bears, *Mycobacterium bovis*, Polymerase Chain Reaction

INTRODUCTION

Tuberculosis is a common disease of wild and domestic animals particularly those kept under captive situation like zoological setup where they are closely associated with the animal keepers and personnel involved in day-to-day activities (Winkler and Gale 1970; Thoen and steele 2006). Tuberculosis caused by bacteria of the *Mycobacterium tuberculosis* complex, including *M.tuberculosis*, *M.bovis*, *M.africanum* and other mycobacterial species (Cousins et al 1991). However, *M.bovis* is most common mammalian tuberculosis problem in zoological parks (Stetter et al 1995). Tuberculosis has been reported in many species of wild mammals and birds in captivity (Sen Gupta, 1974; Rathore and Kher, 1982). Though carnivores resistant to tuberculosis it have still been reported in lion, tigers, leopards, caracals and bears (Das and Jayarao, 1986; Arora, 1994). As far as sloth bear concerned, there are only few reports available (Rathore and Kher, 1982; Muralimanohar, 2006). Also in zoological setup lack of reliable diagnostic test to make tuberculosis is a serious threat to human and other animals (Stetter et al 1995). Since the present focus is on the use of modern technique for disease investigation, faecal samples of sloth bear were screened for the evidence of *M.bovis* using polymerase chain reaction (PCR). In the present study *pncA* gene that is responsible for this property was selected for diagnosing *M.bovis* in sloth bears using polymerase sequence specific to *pncA* gene.

MATERIALS AND METHODS

Samples and Genomic DNA Isolation

Forty two apparently healthy animals between 2.5 to 12 years of age were chosen for this study from Bannerghatta Biological Park, Bangalore. Five gram of faecal samples were collected from each animal in sterile container and immediately stored at 2.5°C. DNA was extracted as per the (Ultraclean™ faecal DNA kit, MoBio laboratories Inc.) manufacturer's protocol.

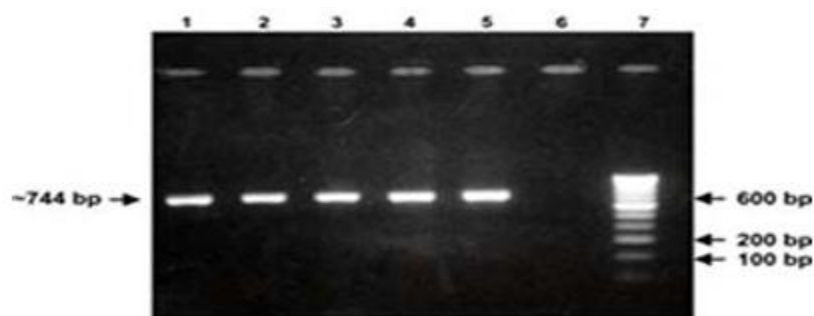
Polymerase Chain Reaction Amplification and Condition

The genomic DNA thus extracted after amplified using entire *pncA* open reading frame, as well as 124 bp of upstream and 54 bp of downstream sequence. A 744 bp PCR product was generated using the primers *pncA*-8 (5'-GGTTGGGTGGCCGCCGTCAG-3') and *pncA*-11 (5'-GCTTTGCGGCGAGCGCTCCA-3'). The *pncA* open reading frames (561 bp) begin at nucleotide 125 of the 744 bp PCR product and ends at nucleotide 685.

The PCR reactions were performed in volumes of 25µl, each containing 12.5µl of PCR master mix (2.0x) (Eppendorf AG, 22331 Hamburg, Germany), 0.5µl of each primer (forward and reverse), 2µl of ample DNA and 9.5 µl of distilled water. Amplification was performed in an Eppendorf Mastercycler Gradient as suggested by Hannan et al (2001) with slight modifications. The amplification was carried out with an initial denaturation at 94°C for 5 minutes followed by denaturation at 94°C at 1 minute, annealing at 55°C for 1 minute and extension at 72°C for 2 minutes for 35 cycles. The final elongation was carried out at 72°C for 10 minutes followed by hold at 4°C. PCR products were electrophoresed along with a 100 bp ladder (Bangalore Genei) on 1.5% agarose gel and stained with ethidium bromide. Images were captured in a gel documentation system (Vilber lourmat, Germany).

RESULTS AND DISCUSSIONS

Out of 42 faecal samples, 5 samples yielded on expected amplified PCR product size of 744 bp when electrophoresed in 1.5% agarose gel (Figure 1). This 744 bp product that was generated using the *pncA* 8 and *pncA* 11 primers agreed with the other experts (Hannan et al 2001). Genotypic identification of *M.bovis* species can be performed by identifying the *pncA* gene of *M.bovis* that will distinguish it from other members of *M.tuberculosis* complex. The result indicates that the 744 bp *pncA* amplicon is very characteristic of *M.bovis*. The study shows that *pncA* gene is well suited in diagnostic point of view for bovine tuberculosis diagnosis in wild animals.



Lane 1 to 6: Test samples
 Lane 1 to 5: Positive for *M.bovis*
 Lane 6: Negative for *M.bovis*
 Lane 7: 100bp size DNA markers

Figure 1: Amplified 744 bp Products of Different Faecal Samples using *M. bovis* Specific Gene *pncA*

The result of this study highlighted that tuberculosis can be caused by *M.bovis* in sloth bears, this was in agreement with Muralimanohar et al (2006), who had earlier reported tuberculosis in sloth bears. Most of the sloth bears in this study had been rescued from various places, where they had earlier been kept under shabby, stressful, starved conditions and could have probably picked up infection from other domestic animals and humans. Thakuria (1996) was also of the opinion that healthy animals can become infected through contamination of food and water by fecal material, urine and exudates of diseased animals. These bears were also positive for severe worm burden that could have made them more susceptible to diseases as supported by Berland (1982).

Since clinical signs are not evident at the early stage of infection (Thoen, 1988), it was of the opinion that PCR assay could help in early diagnosis of the clinical specimen and environmental samples as reported by Hawkey et al (1996). However reports regarding the diagnosis of Mycobacterial infection using faecal samples are scarce. Balamurugan et al (2006) used faecal samples for detection of intestinal tuberculosis among humans using PCR assay. It may be concluded that PCR is a rapid diagnostic method for detection of *Mycobacterium bovis* in bears using faecal samples. Hence, such rapid diagnostic tests could help in early detection, isolation and treatment which otherwise could be life threatening to human beings.

ACKNOWLEDGEMENTS

The authors are thankful to PCCF and Chief Wildlife Warden, Bangalore and Secretary Wildlife SOS® for granting permission to carryout this research work. Facilities provided by Department of Animal Biotechnology, Madras veterinary College is also duly acknowledged.

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